

Biological relevance of polyploidy: ecology to genomics

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The role of triploid hybrids in the evolutionary dynamics of mixed-ploidy populations

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Theory suggests that the evolution of autotetraploids within diploid populations will be opposed by a minority-cytotype mating disadvantage. The role of triploids in promoting autotetraploid establishment is rarely considered, yet triploids are often found in natural populations and are formed in experimental crosses. Here, I evaluate the effects of triploids on autotetraploid evolution using computer simulations and by synthesizing research on the evolutionary dynamics of mixed-ploidy populations in *Chamerion angustifolium* (Onagraceae). Simulations show that the fate of a tetraploid in a diploid population varies qualitatively depending on the relative fitness of triploids, the ploidy of their gametes and the fitness of diploids relative to tetraploids. In general, even partially fit triploids can increase the likelihood of diploid–tetraploid coexistence and, in some cases, facilitate tetraploid fixation. Within the diploid–tetraploid contact zone of *C. angustifolium*, mixed populations are common (43%), and often (39%) contain triploids. Greenhouse and field studies indicate that triploid fitness is low (9% of diploids) but variable. Furthermore, euploid gametes produced by triploids can be x , $2x$ or $3x$ and contribute the majority (62%) of new polyploids formed in each generation (2.3×10^{-3}). Although triploid bridge, alone, may not account for the evolution of autotetraploidy in *C. angustifolium*, it probably contributes to the prevalence of mixed-ploidy populations in this species. Therefore, in contrast to hybrids in homoploid species, triploids may actually facilitate rather than diminish the fixation of tetraploids by enhancing the rate of formation. © 2004 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2004, **82**, 537–546.

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INTRODUCTION

Most biologists would agree that polyploidy has a wide taxonomic distribution and that the transition from diploidy to polyploidy has occurred numerous times, even within individual taxa (Soltis & Soltis, 1999). However, it is still unclear precisely how polyploidy evolves; that is, by what processes polyploids arise, establish and persist in natural populations (Thompson & Lumaret, 1992; and Abbott & Lowe, 2004; Ainouche, Baume & Salmon, 2004; Soltis *et al.*, 2004 – all this issue). Over the last 15 years, population biologists have been exploring this question by examining

the rates and mechanisms of polyploid formation (Sato *et al.*, 1993; Ramsey & Schemske, 1998) and the factors influencing their establishment (Lumaret *et al.*, 1987; Maceira, Jacquard & Lumaret, 1993; Bretagnolle & Thompson, 1996; Petit, Bretagnolle & Felber, 1999; Ramsey & Schemske, 2002; Thompson, Nuismer & Merg, 2004 – this issue) in natural populations.

Polyploid evolution has been examined in several theoretical models, all of which evaluate the fate of autotetraploids that arise within populations of their diploid progenitors (Levin, 1975; Fowler & Levin, 1984; Felber, 1991; Rodriguez, 1996). Although this scenario may be an oversimplification of many extant species, it does offer some general insights into the initial stages of polyploid evolution. The most complete

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models include a mechanism for tetraploid formation through the union of unreduced ($n = 2n$) gametes as well as some consideration of the factors affecting diploid and tetraploid establishment, such as their viability and fertility. Under this theoretical framework, the dynamics of mixed-ploidy populations are dominated by a frequency-dependent process called minority cytotype disadvantage, in which the rare ploidy (diploid or tetraploid) experiences a transmission disadvantage (Levin, 1975; Felber, 1991). This reduced fitness is a consequence of the high proportion of hybrid matings experienced by rare cytotypes and the strong genetic incompatibility between diploids and polyploids (Levin, 1975). The minority disadvantage has been demonstrated empirically (Husband, 2000) and, yet, is at odds with the broad taxonomic distribution of polyploidy (including allopolyploids and increasingly autopolyploids) and its prevalence in groups such as the angiosperms and ferns. Thus, understanding the conditions for the establishment of polyploids remains a primary focus in population biology. Theoretical research indicates that a range of ecological (autogamy, niche differentiation, small populations) and genetic (flowering time differentiation, fitness differences, $2n$ gametes) factors may contribute to establishment; however, few of these have been tested empirically in a systematic or comprehensive manner [although see research on *Dactylis glomerata* (Lumaret *et al.*, 1987), *Mercurialis annua* (Pannell, Obbard & Buggs, 2004 – this issue) and *Chamerion angustifolium* (this paper)].

POTENTIAL ROLE OF TRIPLOIDS

With one exception (Felber & Bever, 1997), all polyploid models make the starting assumption that triploids, formed from the union of $n = 2x$ and $n = x$ gametes, are lethal. This reflects a widely held view that diploids and their polyploid derivatives are isolated by strong, post-zygotic genetic barriers (Futuyma, 1998; Schluter, 2001). However, this assumption is not entirely supported by the literature on wild species of mixed ploidy, and is probably an oversimplification. For example, in a survey of 249 species of angiosperms that comprised both diploid and tetraploid individuals (or higher ploidies), 39 (16%) contained some triploids (B. Mable, unpubl. data). In a smaller subset of mixed-ploidy plant species, for which extensive geographical surveys have been conducted, triploids (or odd-numbered hybrid cytotypes) were observed in nine cases (45%, Table 1), albeit often at low frequencies. Of particular note is the high incidence of triploids in species such as *Galax urceolata* (Burton & Husband, 1999) and *C. angustifolium* (Husband & Schemske, 1998) in which sampling has been

intense and over a wide geographical range. In addition, diploids and polyploids in these species regularly occur in sympatry. In two other species, for which triploids have not been observed, diploid–polyploid hybrids were produced in experimental crosses. Similar patterns were reported by Ramsey & Schemske (1998) for wild and domesticated species, with triploids produced in 44% of the between-ploidy crosses examined.

Additional motivation to study triploids has come from my own work on the evolutionary dynamics of diploids and autotetraploids in the perennial, herbaceous plant *Chamerion angustifolium* L. Holub (Onagraceae). This species has a circumpolar distribution and comprises diploids ($2n = 2x = 36$), autotetraploids ($2n = 4x = 72$) and, rarely, autohexaploids ($2n = 6x = 108$) (Mosquin, 1967). In North America, diploid *C. angustifolium* occurs in northern latitudes, whereas tetraploids are found in the south. My research has focused on the overlap zone between these ploidies, which follows the southern limits of the boreal forest and along the Rocky Mountains, because it provides an opportunity to examine the interactions between diploids and tetraploids within mixed-ploidy populations.

We have conducted geographical surveys of over 66 populations throughout the American and Canadian Rocky Mountains (Husband & Schemske, 1998; H. Sabara & B. C. Husband, unpubl. data). In contrast to more cursory surveys in other species, we found that 68% of all populations contained more than one ploidy and 43% contained both diploids and tetraploids in moderate frequencies (0.2–0.4). Perhaps most striking was that 39% of these evenly mixed populations contained triploids, which ranged in frequency from 1 to 20% (H. Sabara & B. C. Husband, unpubl. data) and occurred in close proximity to the other cytotypes. The high incidence of triploids is at odds with the general assumption of strong triploid block and raises questions about the role they play, if any, in the evolutionary dynamics of these populations.

Triploids may influence autotetraploid establishment by providing an additional pathway of tetraploid formation. Tetraploids form through a variety of routes (Harlan & de Wet, 1975; Ramsey & Schemske, 1998), one of which is thought to involve unreduced gametes ($n = 2x = 2n$ gametes), which contain the somatic chromosome number (Bretagnolle & Thompson, 1995; Ramsey & Schemske, 1998). The conventional means for autotetraploid formation involves the union of two unreduced ($n = 2x = 2n$) gametes in a single step (Type I pathway). Because this mechanism would occur with the joint probability of two unlikely events, it is considered quite rare in natural populations. Alternatively, tetraploids may be produced in two steps, via a triploid intermediary, through a pro-

Table 1. Summary of the incidence of odd-numbered, diploid–polyploid hybrids in wild species of mixed ploidy. Observations noted for field and experimental crosses (–, no hybrids; +, hybrids; blanks = absence of information)

Species	Ploidy	Hybrids		Reference
		Field	Crosses	
<i>Centaurea jacea</i>	2x,4x	–	+	(1)
<i>Chamerion angustifolium</i>	2x,3x,4x	+	+	(2,3)
<i>Galax urceolata</i>	2x,3x,4x	+		(4)
<i>Heuchera grossularifolia</i>	2x,3x,4x	+		(5,6)
<i>Plantago media</i>	2x,3x,4x	+		(7)
<i>Dactylis glomerata</i>	2x,3x,4x	–	+	(8,18)
<i>Claytonia virginica</i>	2x,3x,5x,4x	+		(9)
<i>Fragaria</i>	2x,8x	–		(10)
<i>Deschampsia cespitosa</i>	2x,4x	–		(11)
<i>Agrostis stolonifera</i>	4x,5x,6x	+		(12)
<i>Lathyrus pratensis</i>	2x,4x	–		(13)
<i>Tripleurospermum inodorum</i>	2x,4x	–	+	(14)
<i>Anthoxanthum odouratum</i>	2x,3x,4x	+		(15)
<i>Andropogon gerardii</i>	6x,7x, 8x	++		(16,17)
<i>Lotus alpinus/corniculata</i>	2x,4x	–		(19)
<i>Eriophyllum confertiflorum</i>	2x,4x,6x,8x	–	+	(20)
<i>Heuchera micrantha</i>	2x,4x	–		(21)
<i>Biscutella laevigata</i>	2x,4x	–		(22)
<i>Tolmiea menziesii</i>	2x,4x	–		(23)
<i>Fraxinus americana</i>	2x,4x,6x	+		(24)

(1) Hardy *et al.* (2000); (2) Husband & Schemske (1998); (3) H. Sabara & B. C. Husband (unpubl. data); (4) Burton & Husband (1999); (5) Thompson *et al.* (1997); (6) Wolf, Soltis & Soltis (1990); (7) Van Dijk, Hartog & Van Delden (1992); (8) Lumaret *et al.* (1987) (9) Lewis & Suda (1976); (10) Hancock & Brunghurst (1981); (11) Rothera & Davy (1986); (12) Kik, Linders & Bijlsma (1992); (13) Brunsberg (1977); (14) Kay (1969); (15) cited as Felber, in press, in Felber & Bever (1997); (16) Keeler & Davis (1999); (17) Keeler (1992); (18) van Santen, Hugessen & Casler (1991); (19) Jay (1991); (20) Mooring (1994); (21) Ness, Soltis & Soltis (1989); (22) Manton (1934); (23) Soltis (1984); (24) Black & Beckmann (1983).

cess called triploid bridge (Type II pathway). Here, triploids are produced through the union of x and $2x$ gametes. Depending on their fertility and the ploidy of their functional gametes (e.g. $n = x$, $2x$ or $3x$), triploids may produce tetraploid offspring through backcrosses with diploids or matings with other triploids.

By influencing the rate of polyploid formation, triploids may enhance the probability of tetraploid establishment. Felber's (1991) theoretical studies showed how recurrent production of tetraploids can result in the formation of a dynamic equilibrium, with diploids in the majority and tetraploids in the minority. Furthermore, if the rate of formation is sufficiently high, the frequency of polyploids may cross a threshold, thereby overcoming minority cytotype disadvantage, which allows polyploids to spread to fixation. Although this idea is not new, in theory, it has not been explored empirically by population biologists, probably because of a lack of appropriate information for natural populations and no clear sense of the relative importance of triploids when they are rare or only partially fertile.

Here I evaluate the effects of triploids on the spread and establishment of autotetraploids within diploid populations. First, I describe the results from computer simulations designed to examine the impact of triploid fitness and gamete ploidy on tetraploid establishment. This extends the work of Felber & Bever (1997) to include a range of gamete ploidies for triploids. Second, I explore the impact of triploids on mixed-ploidy populations of *C. angustifolium*, a species for which most of the critical model parameters have been estimated.

SIMULATIONS OF TRIPLOIDS IN MIXED-PLOIDY POPULATIONS

Three factors may influence the impact of triploids on tetraploid establishment: their rate of formation (or, conversely, the strength of triploid block), their fitness (viability and fertility) relative to diploids and tetraploids, and the ploidy of their functional gametes. Studies of triploids produced by crossing

diploids and tetraploids indicate that triploids have lower viability and higher rates of sterility due to aneuploidy; however, their fitnesses are frequently non-zero (Ramsey & Schemske, 1998). Furthermore, the viable gametes they do produce may be monoploid ($n = x$), diploid ($n = 2x$) or unreduced ($n = 3x$) (Ramsey & Schemske, 1998). Here, I used individual-based simulations to examine the effect of these triploid characteristics on the likelihood that a rare tetraploid can be maintained or spread to fixation within a diploid population.

Each simulated population consisted of 1000 individuals with non-overlapping generations. Initially, all populations were completely diploid; however, triploids and tetraploids could arise recurrently through the union of unreduced gametes. The default frequency of unreduced gametes in diploids was set to 0.03, based on estimates from *C. angustifolium* (Burton & Husband, 2001). At each generation, the individuals were subject to selective mortality as determined by their ploidy-specific relative fitnesses and their frequencies standardized to one. After selection, offspring for the next generation were generated through a mating phase, wherein $n = 1000$ pairs of individuals were drawn at random from the population to serve as parents, each of a single progeny. No assortative mating was included in the model. The ploidy of the offspring was determined according to the ploidy probability distributions for different parental combinations. For example, if the parental pair consisted of two diploids, the likelihood of having a diploid offspring would be proportional to the squared frequency of reduced gametes ($n = x$) produced by diploids; triploid offspring would occur at the rate of two times the frequency of reduced gametes multiplied by the rate of unreduced gametes ($n = 2x = 2n$); and tetraploid offspring would occur at a rate equivalent to the square of the rate of unreduced gamete production. I assumed that tetraploids produced only $n = 2x$ pollen, and $2n = 5x$ or $6x$ offspring are not viable. Each simulation was run for 1000 generations and was replicated 100 times to allow for stochastic variation.

Plant species differ with respect to the ploidy of gametes produced by triploids, yet current theoretical models do not explore the impact of this attribute on tetraploid establishment. In this simulation, I explored three different gamete ploidy models, where triploids produce: (i) haploid ($n = x$) and diploid ($n = 2x$) gametes in equal proportions; (ii) haploid, diploid and unreduced gametes ($n = 3x$) in equal proportions; and (iii) only unreduced ($n = 3x$) gametes. Each model was examined for a range of triploid relative fitnesses ($w_{3x} = 0.0$ – 1 , at 0.1 intervals) and when tetraploid fitness was either equal to diploids ($w_{2x} = w_{4x} = 1$) or half that of diploids ($w_{2x} \times 0.5 = w_{4x}$). The impact of

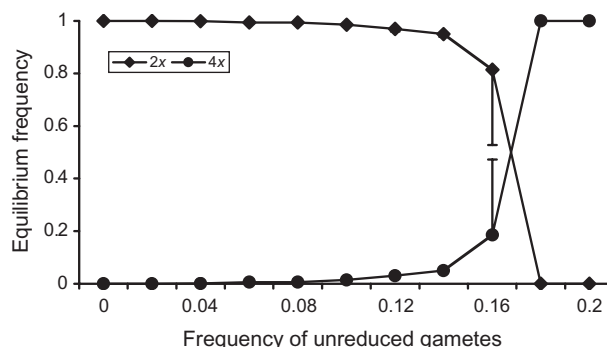


Figure 1. Equilibrium frequencies of tetraploids and diploids as a function of unreduced gamete production in diploids. All simulations assume that triploid fitness is zero. Standard deviations are included for all points, but are not visible in most cases.

triploids was assessed in two ways: by determining the frequencies of diploids, triploids and tetraploids at equilibrium, and the rate of unreduced gamete production in diploids that is necessary for tetraploids to spread to fixation.

In the absence of triploids (i.e. $w_{3x} = 0$), the simulation results were identical to previous models (Felber, 1991). Specifically, when no unreduced gametes are produced by diploids, tetraploids were unable to invade diploid populations because no tetraploids were being formed (Fig. 1). Increasing the frequency of unreduced gametes produced by diploids above zero resulted in a stable coexistence between diploids and tetraploids, with tetraploids in the minority (Fig. 1). Tetraploids did not spread to fixation under these conditions due to minority cytotype disadvantage. However, once the frequency of unreduced gametes in diploids exceeded ~17%, tetraploid formation was high enough to allow them to spread and replace diploids (Fig. 1).

Increasing the relative fitness of triploids above zero and setting the fitness of tetraploids equal to diploids enhanced the likelihood that tetraploids would persist at equilibrium (Fig. 2A, B). At low triploid fitnesses, tetraploids were maintained at low frequency, along with triploids (data not shown). Above a critical triploid fitness, however, tetraploids would spread to fixation. The point at which this occurred depended on the ploidy of the gametes produced by triploids. When the functional gametes of triploid individuals were all $3x$ (i.e. unreduced), tetraploid fixation occurred at relatively low fitness values (0.2). In comparison, the fitness threshold was 0.4 when triploids produced $x, 2x$ and $3x$ gametes and 0.8 with only $x, 2x$ gametes. In the latter scenario, increasing the fitness of triploids beyond 0.9 resulted in a progressive decline in the likelihood of tetraploid fixation. This result was shown

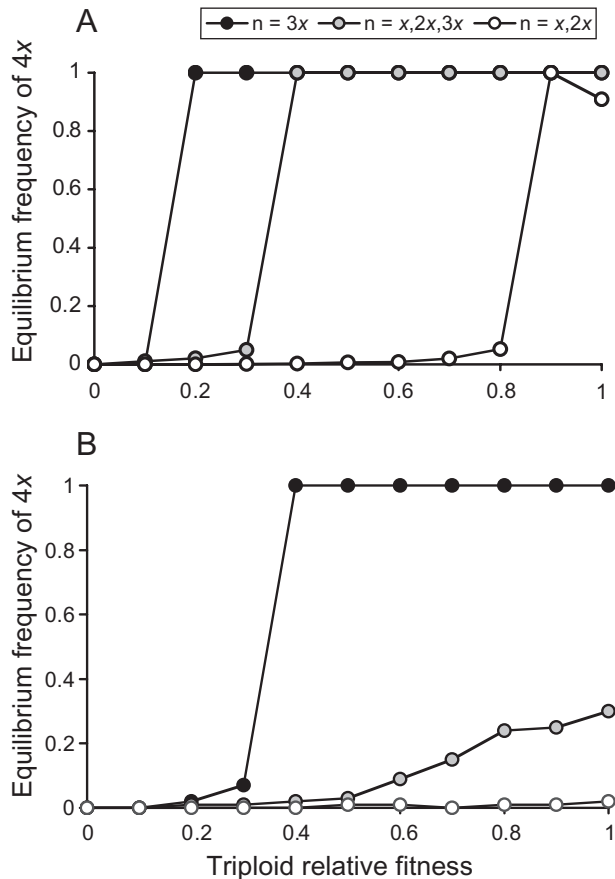


Figure 2. Equilibrium frequency of tetraploids in simulated populations as a function of triploid fitness when (A) diploids and tetraploids have equal fitnesses, or (B) tetraploids have half the fitness of diploids. All populations were initially diploid. Diploids produced 3% unreduced gametes. Lines represent three gamete compositions: $3x$ gametes only (solid circles); x , $2x$, and $3x$ gametes (grey circles) and x and $2x$ gametes (open circles). Frequencies of diploids and triploids not shown.

by Felber & Bever (1997) to occur only when tetraploid fitness was less than diploids. In this study, when the fitness of tetraploids was half that of diploids, the presence of triploids resulted in increased persistence of tetraploids. However, in contrast to when $w_{2x} = w_{4x}$, tetraploids coexisted over a larger range of triploid fitnesses and tetraploid fixation was only observed when triploids produced $3x$ gametes (Fig. 2B).

Expressed in another way, the frequency of unreduced gametes in diploids that is necessary for tetraploid fixation decreased when triploids had a non-zero fitness (Fig. 3, results shown for $w_{3x} = 0.1$). When $w_{2x} = w_{4x}$, the critical rate of unreduced gamete production was 16% when triploids produced $x, 2x$ gametes, 12% with $x, 2x, 3x$ and 8% when only unreduced gametes ($n = 3x$) were produced (Fig. 3). The

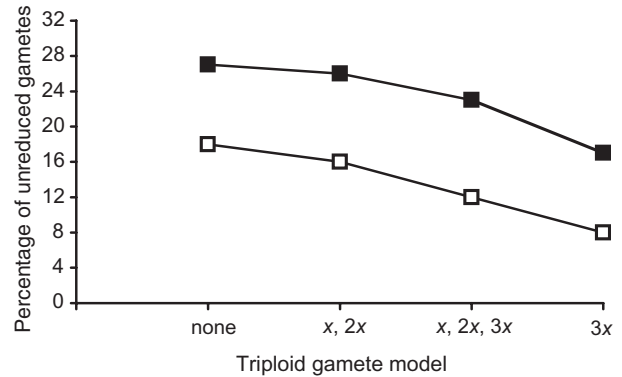


Figure 3. Mean rate of unreduced gamete production in diploids that is necessary for tetraploids to arise and spread to fixation. The rate is given for four different models of gamete production by triploids (0, no gametes; 1, gametes are $n = 3x$; 2, gametes are $n = x, 2x$ and $3x$; 3, gametes are $n = x$ and $2x$). These results are given for diploids and tetraploids with equal fitnesses (open squares) and when tetraploids are half as fit (solid squares).

trend was similar when $w_{4x} = 0.5w_{2x}$, although the absolute values were higher (Fig. 3).

I have only shown the results for the case in which diploids produce unreduced gametes at a rate of 3%. Clearly, this value will vary among species. Theoretically, increasing or decreasing the rate will alter the absolute values shown here, but, qualitatively, I expect the effects of triploids to remain the same. Similarly, increasing the rate of assortative mating in populations should not affect the equilibrium frequencies observed, just the rate at which they are achieved. Because assortative mating effectively weakens the impact of minority cytotype exclusion, it will increase the time to equilibrium (Levin, 1975).

The simulations offer a number of important lessons. First, a low rate of unreduced gamete production by diploids can lead to the infrequent production of tetraploids and triploids, and thus establish the conditions for a stable coexistence between cytotypes (also see Felber & Bever, 1997). Second, viable triploids enhance the likelihood that tetraploids will persist or even spread to fixation, but the extent of this effect depends on the ploidy of the gametes that triploids produce and the fitness of tetraploids relative to diploids. Specifically, the presence of unreduced gametes ($n = 3x$) in triploids has a greater promoting effect than the other two models, presumably because it effectively increases the rate of tetraploid formation each generation and thereby pushes the population closer to the frequency threshold. Finally, although the impact of triploids tends to increase with relative fitness, it is important to acknowledge that its influence on tetraploid persistence can be quite strong even

when triploids are relatively unfit. This is particularly true when most gametes are unreduced rather than monoploid or diploid.

TRIPLOID BRIDGE IN *C. ANGUSTIFOLIUM*

Despite the potential for triploids to promote the establishment of tetraploids within populations of their diploid progenitor (Harlan & de Wet, 1975; Felber, 1991), the absence of data on the critical parameters for any single species has precluded a definitive evaluation of triploids in natural populations (Felber & Bever, 1997). Recent research on the evolutionary dynamics of the diploid–tetraploid contact zone in *C. angustifolium* (Onagraceae) offers such an opportunity. Earlier, I described the prevalence of triploids within mixed-ploidy populations of this species. Here, I synthesize results on the major components of triploid bridge, including: likelihood of triploid formation, triploid viability and fertility, gamete composition and ploidy of offspring produced by triploid parents. I then incorporate this information into a simulation to explore the impact of triploid bridge in this species.

To evaluate the role of triploid bridge in mixed-ploidy populations of this species, Burton & Husband (2000) first examined the rate of triploid formation in $2x \times 4x$ crosses. In an experiment with several diploid–tetraploid pairs, raised from open-pollinated seed from the overlap zone, they crossed each member of a pair with each other as well as to a plant of the same ploidy to generate $2x \times 2x$, $2x \times 4x$, $4x \times 2x$ and $4x \times 4x$ offspring. The likelihood of triploid formation was estimated as seed production in the reciprocal $2x \times 4x$ crosses. Seed number in $2x \times 4x$ crosses was 49% of that seen in $2x \times 2x$ crosses, suggesting that triploid block ($1 - w_{3x} = 0.51$) is relatively weak in this species (Fig. 4). Ramsey & Schemske (1998) suggested that successful triploid formation may be more likely in species lacking endosperm in the mature seed. *C. angustifolium* is consistent with that idea. It is also interesting to note that there were no differences between reciprocal $2x \times 4x$ crosses. Triploid block is viewed as the result of a ploidy imbalance between endosperm and embryo, which normally differs among reciprocal crosses. However, like other members of the Onagraceae, *C. angustifolium* has a monosporic type of embryo sac development, which means that the ploidy ratio (1 : 1) is the same in both reciprocal crosses. Alternatively, the genome imprinting hypothesis argues that triploid fitness is a product of maternal–paternal ploidy ratios (Haig & Westoby, 1991). These ratios do differ among reciprocal crosses in *C. angustifolium*, yet no differences in seed production were observed (Burton & Husband, 2000).

Once produced, the fate of triploids will depend on their fitness relative to diploids and tetraploids. In the

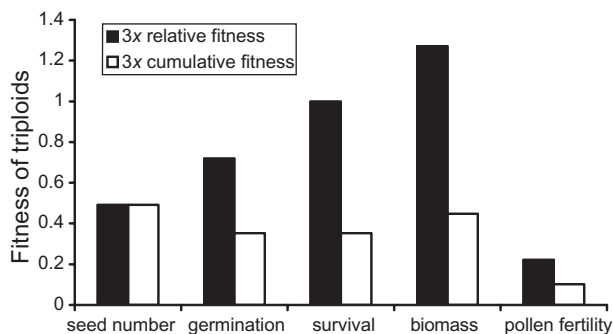


Figure 4. Relative fitness (by stage) and cumulative fitness of triploids ($2x \times 4x$ crosses) compared with diploids for five successive life stages in *Chamerion angustifolium*, based on a greenhouse study (Burton & Husband, 2000). Seed number refers to the fertility of the cross, not to seed number of triploid plants.

greenhouse, Burton & Husband (2000) compared the survival and growth of offspring generated from experimental crosses in a 9-week growth experiment. Triploid progeny from $2x \times 4x$ crosses had lower mean seed germination than diploid ($2x \times 2x$) and tetraploid ($4x \times 4x$) offspring, and survival of seedlings to flowering did not differ among cross types (Fig. 4). Combining data on germination and survival in a multiplicative fitness function found that mean viability fitness of triploids was 72% of diploids.

We have also inferred the viability of triploids in the field by comparing the frequencies of triploids in open-pollinated seed to that in the adult stage in several populations (H. Sabara & B. C. Husband, unpubl. data). Although this method makes the untested assumption that populations have approached a stable equilibrium with respect to ploidy, it has the benefit of providing realized measures of fitness during germination and seedling establishment life stages. Currently, we have screened progeny and adults from nine mixed populations in the Rocky Mountains ($n = 1638$ offspring; $n = 305$ adults). Triploid frequencies in the offspring exceeded that in the adult stage in seven of nine populations (one was the reverse, in the other there was no difference). The relative fitness of triploids ranged from 0 to 2.4 among populations, with a mean of 0.75. Although the variance is high, the mean value is similar to the estimate from the greenhouse experiment, and confirms that the viability of triploids is low but not zero.

The fertility of triploids was estimated as the product of flower production, measured using biomass as a correlate, and pollen fertility (number of viable grains produced). Unlike any other fitness component examined, the biomass of triploids was, in fact, significantly higher than in diploids (Fig. 4), and there were no dif-

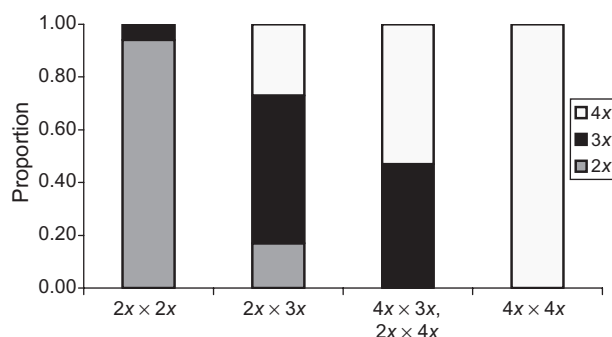


Figure 5. Proportions of diploid (grey), triploid (black) and tetraploid (white) offspring produced in five different between-ploidy crosses. Cross-type with similar offspring composition are pooled. Redrawn and modified from Burton & Husband (2001).

ferences among reciprocal crosses. By contrast, pollen fertility was only 22% of that in diploids, marking the biggest deviation of any fitness component examined. This difference was more the result of low pollen viability than low pollen production. In addition, there were large differences with respect to pollen fertility between reciprocal crosses; triploids with tetraploid mothers had particularly low fertilities.

Overall, the cumulative effects of between-ploidy crosses on seed maturation, germination, survival, biomass and pollen fertility were quite large. Triploids had a cumulative fitness of only 10% of the diploids (Fig. 4), whereas tetraploid fitness was 61% (Burton & Husband, 2000). Nevertheless, theoretical studies indicate that such low fitnesses can still have a significant impact on tetraploid establishment by maintaining tetraploids in a stable coexistence with diploids. Furthermore, if the majority of gametes produced by triploids are unreduced, tetraploid fixation can still be achieved at such low triploid fitnesses. It is also important to consider that from our studies, triploid viability may vary widely among populations and therefore may well exceed the 10% value in some circumstances.

The final information required to assess the role of triploids in *C. angustifolium* is the ploidy of offspring of triploid and diploid parents. This was assessed through a crossing experiment that involved diploids, triploids and tetraploids (Burton & Husband, 2001). From these crosses, we assessed the fecundity of the various parental combinations as well as the ploidy of the surviving offspring (inferred from DNA content). As the different pollen fertilities would have predicted, fecundity differed widely among the ploidy combinations. Diploid × diploid crosses yielded the highest seedset. Crosses involving one or more triploid parents had relatively low fecundity (<10% seed set), except for the 3x × 4x cross, which was similar to 4x × 4x and 4x × 2x crosses (10% > seed set <35%).

The ploidy of offspring differed significantly among the major cross types (Fig. 5). For example, the majority (94%) of offspring from 2x × 2x crosses were, in fact, diploid, although there was a minority of triploids (6%). Based on a separate screening of a larger number of offspring ($n = 650$ plants) from 2x × 2x crosses, we also found a small frequency (0.006) of tetraploid offspring. From this analysis, we infer that 2–3% of gametes produced by diploids are unreduced. Furthermore, a recent analysis of unreduced gamete frequencies measured directly from the DNA content of pollen, using flow cytometry, is of the same order of magnitude (P. Kron & B. C. Husband, unpubl. data). Interestingly, the estimate for this species is roughly ten times higher than the estimate by Ramsey & Schemske (1998), based largely on crop plants.

Offspring generated from 2x × 3x crosses were either diploid, triploid or tetraploid (Fig. 5). Assuming that diploids produce few if any unreduced gametes, these results are consistent with triploids producing a combination of monoploid ($n = x$, 17%) diploid ($n = 2x$, 56%) and unreduced gametes ($n = 3x$, 27%). Most importantly, this suggests that tetraploids can be produced *de novo* through triploid bridge; in fact, 81% of all gametes will contribute to the production of tetraploids or more triploids. In addition, crosses between triploids and tetraploids resulted in triploid and tetraploid offspring (as well as some putative pentaploids). Crosses among tetraploids produced tetraploids; however, this result has varied among different experiments (including that reported by Burton & Husband, 2001).

So, what impact do triploids have on tetraploid establishment in *C. angustifolium*. To consider this question, I first estimated the rate of tetraploid formation and the contribution of triploid bridge to this process. Then I explored the long-term impacts in a final simulation. The rate of *de novo* tetraploid formation was estimated for the first generations in a completely diploid population, following Ramsey & Schemske (1998). Specifically, the rate of tetraploid production via the Type I pathway (i.e. union of two unreduced gametes) is simply the square of the frequency of unreduced gametes in diploids ($0.03 \times 0.03 = 0.0009$). The rate of formation via the triploid bridge (Type II pathway) is the product of (i) the probability of triploid formation, which is $2 \times \text{freq}(2n \text{ gametes} = 0.03) \times \text{freq}(n \text{ gametes} = 0.97) = 0.0582$, (ii) the relative viability and fertility of triploids ($w_{3x} = 0.09$) and (iii) the likelihood of tetraploid offspring (0.279, assuming random outcrossing, 0.405 through triploid–triploid matings, 0.286 through random mating, which includes selfing). In *C. angustifolium* this amounts to 1.5×10^{-3} tetraploids per generation via the triploid bridge (under random mating) and 9×10^{-4} from diploids directly for a total of 2.4×10^{-3} tetraploids per generation. In other

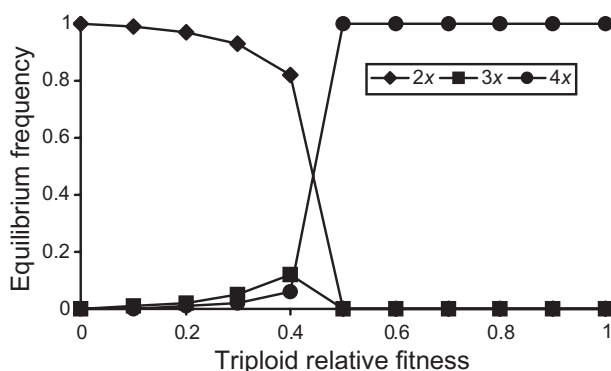


Figure 6. Equilibrium frequencies of diploids (diamonds), triploids (squares) and tetraploids (circles) expressed as a function of the relative fitness of triploids. All other parameters were set to values estimated previously in *Chamerion angustifolium*. Mean triploid fitness has been estimated for *C. angustifolium* at 0.10, but probably varies with environment.

words, the triploid bridge contributes 62% of the tetraploids produced each generation.

The impact of triploids on the long-term evolution of tetraploids in diploid populations of *C. angustifolium* can be illustrated using a simulation approach. Here, we used the basic simulation framework described above, substituting our species-specific estimates for gamete ploidies, and viabilities and fertilities for diploids, triploids and tetraploids. Because triploid fitness appears to differ widely among populations, we determined the equilibrium frequencies for each ploidy over a range of triploid fitnesses (Fig. 6). As before, when triploids are lethal, tetraploids were unable to establish or persist in diploid populations. As triploid fitness is increased, however, the equilibrium frequency of diploids begins to decrease and the frequencies of triploids and tetraploids increases. Tetraploids spread to fixation when triploid fitness exceeds 0.4, again demonstrating that triploid bridge can have a significant impact even when triploids are partially fit.

Our estimates of triploid fitness averaged 0.10, suggesting that triploid bridge by itself may be insufficient to drive tetraploids to fixation in populations of *C. angustifolium*. Other factors, not included in these simulations, such as assortative mating (Husband & Schemske, 2000) and inbreeding depression (Husband & Schemske, 1997), both of which favour tetraploids, may further weaken the effects of minority cytotype disadvantage and promote tetraploid establishment. Our field studies also suggest that viability of triploids (and probably fitness of diploids and tetraploids) is in fact highly variable and probably depends on microenvironment. Therefore, it is not inconceivable that the

conditions would arise in some locations where triploids alone may tip the balance in favour of tetraploids.

CONCLUSION

The potential contribution that triploids can make to the formation of tetraploids via the triploid bridge has long been recognized; however, the impact of this process on tetraploid establishment within diploid populations has received little theoretical or empirical investigation. Recent models and simulations presented here show that triploids can support the stable coexistence of diploids and tetraploids over a broad range of conditions and, with high fitness, can even promote the fixation of tetraploids. The latter outcome can occur even when triploids are relatively unfit and is most likely when triploids produce some unreduced ($n = 3x = 2n$) gametes. What remains to be investigated is the relative importance of this process in natural populations. Interestingly, triploids are often reported in species that are polymorphic for ploidy and that occur in mixed-ploidy populations. Whether these triploids are a cause or a consequence of ploidy variation is unclear. In *C. angustifolium*, the data on triploid fitness and gamete composition suggest that, at the very least, triploids are playing an important role in maintaining the current distribution of ploidies within the contact zone.

The evidence on triploids collected so far suggests an important role for hybrids in polyploid speciation, a role that may be fundamentally different than with homoploids. In homoploid systems, hybrids are generally viewed as a homogenizing force that prevents the divergence of sympatric populations. By contrast, it is the presence of diploid–polyploid hybrids that allows polyploids to overcome their minority disadvantage and ultimately to establishment in the presence of their diploid ancestors.

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